Expression, function, and regulation of the *hairy* segmentation protein in the *Drosophila* embryo

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The *Drosophila* pair-rule segmentation gene, *hairy*, is required for the proper development of alternate embryonic segment primordia and the normal spatial expression of another pair-rule gene, *fushi tarazu* (*ftz*). We demonstrate that the product of the *hairy* gene is localized to the nuclei of cells in eight distinct regions of the early embryo. The major stripes of protein accumulation comprise the posterior half and the adjacent anterior portion of alternate segment primordia; the *hairy* pattern overlaps transiently with *ftz* expression in a narrow band of cells. Unlike other pair-rule products that have been studied, *hairy* is not expressed in the embryonic nervous system. The spatial distribution of the nuclear *hairy* protein and the pattern of *ftz* expression in embryos lacking *hairy* function suggests that *hairy* plays a direct role in repressing *ftz* gene expression. The pattern of *hairy* expression in gap gene mutants that affect *ftz* expression indicates that the gap genes largely act indirectly upon *ftz* through their effects on *hairy*.

[**Key Words:** Segmentation; *hairy*; gene regulation; embryogenesis; pattern formation]

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The segmentation of the *Drosophila* embryo depends upon the proper action of a number of genes expressed during oogenesis and the early development of the zygote. Systematic screens for zygotically required loci have identified approximately 20 genes that establish the proper number and orientation of body segments (Nüsslein-Volhard and Wieschaus 1980; Jürgens et al. 1984; Nüsslein-Volhard et al. 1984; Wieschaus et al. 1984). At least three classes of segmentation gene activity may be distinguished, based upon the pattern of segment defects observed in the late embryo (Nüsslein-Volhard and Wieschaus 1980; for review, see Akam 1987; Scott and Carroll 1987). The gap genes affect the development of large, mostly contiguous blocks of segments; the pair-rule genes affect the development of segmental pattern at double-segment intervals; and the segment polarity genes control the development of pattern elements within each segment. Many of the segmentation genes have now been cloned and characterized at the molecular level.

One well-studied segmentation gene is the *fushi tarazu* (*ftz*) pair-rule locus. The *ftz* gene is transcribed in seven transverse stripes encircling the cellular blastoderm-stage embryo (Hafen et al. 1984) that correspond to the positions of the embryonic segment primordia affected in *ftz* mutants (Martinez-Arias and Lawrence 1985). The *ftz* protein accumulates within the nuclei of blastoderm cells and in a subset of cells within the developing nervous system (Carroll and Scott 1985). The

striking and complex pattern of *ftz* expression has prompted the analysis of the factors that are involved in the regulation of the *ftz* gene. To identify putative *trans*-acting regulators of *ftz*, the pattern of *ftz* expression in most known zygotic segmentation mutants was examined (Carroll and Scott 1986). All four gap genes and three of seven pair-rule genes affected the establishment of the normal striped pattern. Although these studies established the formal interactions between segmentation genes and *ftz*, nothing could be discerned about the mechanisms of gene interaction.

Of the known zygotic regulators of *ftz*, one gene in particular, the *hairy* pair-rule locus, appears to exhibit a negative regulatory effect on *ftz* (Carroll and Scott 1986; Howard and Ingham 1986). In embryos lacking *hairy* function, it has been shown that *ftz* expression expands to include most cells in the interstripe region (Carroll and Scott 1986; Howard and Ingham 1986). The *hairy* gene has been cloned (Holmgren 1984; Ish-Horowicz et al. 1985) and the expression of its RNA transcripts has been analyzed (Ingham et al. 1985). The *hairy* transcripts accumulate in seven transverse stripes and in a large anterior dorsal region of the cellular blastoderm-stage embryo; the transverse *hairy* stripes are out of phase with and partly overlap the *ftz* stripes at blastoderm.

We describe the pattern of expression, subcellular localization, and spatial regulation of the *hairy* protein. A *hairy* cDNA clone was expressed in bacteria as a fusion protein with β -galactosidase, and antibodies were raised

and purified that reacted specifically with the *hairy* protein. Immunostaining of whole-mount embryos revealed that *hairy* is found within cell nuclei and is expressed in blastoderm-stage cells and in a few cells surrounding part of the tracheal system formed later in embryogenesis. By comparing *ftz* and *hairy* expression in wild-type and mutant embryos, our studies strongly suggest that *hairy* is a direct regulator of *ftz*-gene expression and that three other previously identified potential regulators of *ftz* act primarily indirectly upon *ftz* through the *hairy* gene. Because, unlike most characterized *Drosophila* regulatory genes (Scott and Carroll 1987), *hairy* does not contain any recognizable nucleic acid binding domains (C. Rushlow, pers. comm.), it is of great interest to learn how it regulates expression of *ftz*.

Results and discussion

Antibodies to hairy reveal a nuclear distribution of the protein in two different spatial patterns during embryogenesis

To study the expression and function of the *hairy* gene product, we have prepared antibodies to a *lacZ*-*hairy* fusion protein. Antibodies were raised in rabbits against the 335-amino-acid full-length *hairy* protein (C. Rushlow, pers. comm.), and *hairy*-specific antibody was purified as described previously (Carroll and Laughon 1987). These antibodies were used to stain fixed whole-mount embryos (Carroll and Scott 1985). Cell labeling was visualized either directly with the chromogenic horseradish peroxidase substrate diaminobenzidine (DAB), or indirectly by observing the quenched pattern of the DNA-binding fluorochrome 4,6-diamidino-2-phenyl-indole (DAPI) caused by deposition of oxidized DAB, a method recently developed by Tim Karr (T. Karr and T. Kornberg, in prep.). This latter method, termed

filtered fluorescence imaging (FFI), is more sensitive than previous immunofluorescence methods and makes it possible to visualize both labeled and unlabeled cells.

As is the case for *hairy* RNA transcripts (Ingham et al. 1985), the *hairy* protein accumulates in eight discrete regions of the embryo (Fig. 1a). At the cellular blastoderm stage of embryogenesis, there are seven transverse stripes of *hairy* protein encircling the embryo and one dorsal patch of *hairy* expression at the extreme anterior. The most anterior *hairy* stripe forms at ~75% egg length (measured from the posterior), which is anterior to the position of the cephalic furrow established during gastrulation. The seven stripes are not of equal size and intensity. The first stripe is significantly wider than the others; this is most pronounced on the ventral surface of the embryo where the stripe includes five to seven nuclei, on the average. The fourth stripe is consistently weaker than the others in all dimensions. On average, on the lateral sides of the embryo the *hairy* stripes are about three cells in width and are separated by four to five unlabeled cells (these unlabeled cells can be seen in Fig. 1b most clearly as white, unquenched DAPI-stained nuclei). The dorsal patch of staining extends along ~20% of the dorsal surface of the embryo (~12–14 cells in diam.) and comprises about 150 cells in total.

The *hairy* labeling is concentrated within the nuclei of cells. At high magnification, the staining appears punctate and resembles the pattern of staining exhibited with chromatin-binding counterstains (Fig. 1c). Although most of the cell volume at this stage of embryogenesis is filled by the nucleus, we do not detect *hairy* staining outside of the nucleus. In contrast, cytoplasmic antigens, such as *Escherichia coli* β -galactosidase expressed under *ftz* control (Hiromi et al. 1985), exhibit no staining within the nucleus (data not shown). We conclude that the *hairy* protein is largely confined to the

Figure 1. Expression of the *hairy* protein in the *Drosophila* embryo. Affinity purified rabbit antibody to the *hairy* protein was used to localize *hairy* antigen in formaldehyde-fixed, devitelinized whole-mount embryos. (a) Bright-field image of cellular blastoderm embryo exhibiting eight regions of *hairy* expression. Anterior is to the left, the ventral surface at the bottom. (b) DAPI fluorescent image of the same stained embryo in (a), showing pattern of unlabeled nuclei (bright dots) on background of darkly stained *hairy*-containing nuclei. The deposition of DAB quenches the fluorescence of DAPI (T. Karr and T. Kornberg, in prep.). (c) High-magnification, bright-field view of stripe of *hairy* expression. The labeling appears punctate and is nuclear; the darker areas (arrows) conform to the pattern of chromatin distribution. (d) Later pattern of *hairy* expression at the germ-band-extension stage of embryogenesis. Ten tracheal pits extending from parasegments (PS) 4–13 are clearly labeled, staining appears to be confined to six to eight cells around each pit.

nucleus, consistent with its genetic regulatory function.

The *hairy* protein pattern decays rapidly during gastrulation and germ-band extension. The *hairy* protein is no longer detectable in the stripes shortly after the germ band begins extension. In contrast, the *ftz* and *even-skipped* (*eve*) pair-rule proteins are still detectable at this stage (Carroll and Scott 1985; Frasch et al. 1987). The *hairy* protein is expressed again later in embryogenesis in a group of cells surrounding each of 10 pairs of tracheal pits in the germ-band-extended embryo (Fig. 1d). Only the tracheal pits extending from parasegments 4 to 13 express *hairy*. This transient pattern was not expected from morphological studies of *hairy* mutants; it is not known what function *hairy* has in the six to eight cells stained within each pit. Neither blastoderm nor tracheal pit staining is observed in homozygous *hairy*^{TH94} embryos, indicating that all of the observed staining is due to the bona fide *hairy* product (data not shown). Multiple transcripts of the *hairy* gene have been documented: It is possible that the tracheal pit expression is under different regulatory controls than the blastoderm expression (Ish-Horowicz et al. 1985). Unlike *ftz* and *eve*, *hairy* is not detected within the embryonic ventral nervous system (data not shown). Perhaps the expression patterns of the pair-rule genes in the nervous system represent different functions and regulatory controls of these genes than that which occurs in the ectoderm.

The spatial relationship of hairy expression to other pair-rule genes

Using antibodies to the *eve* (Frasch et al. 1987), *ftz* (Carroll and Scott 1985), and *hairy* products, we have used double labeling of embryos to determine more precisely the spatial relationship between *hairy* and the other pair-rule gene patterns. The *ftz*, *eve*, and *hairy* products all reach their maximum level of accumulation at the completion of cellularization of the blastoderm embryo (Fig. 2a–c). At this moment, each transverse stripe of pair-rule expression is generally narrower than the interstripes. We emphasize that these expression patterns are observed near the completion of cellularization, and the initiation of gastrulation. There are different transient spatial protein patterns during very early cellularization, but they do not represent the final high-level accumulation patterns. At these earlier times, *ftz*, *eve*, and *hairy* all exhibit transient patterns, where different protein stripes vary in width and intensity (T. Karr and T. Kornberg, in prep.; S. Carroll, unpubl.). These transition patterns are striking and could reflect the interactions between segmentation genes in establishing the final patterns. The patterns we detail below demonstrate the outcome of these interactions and help to reveal the regulatory relationships between these three pair-rule genes.

The relatively low level of *hairy* expression made double immunofluorescence localization techniques impossible. Instead, we have visualized protein products simultaneously, using immunoenzymatic techniques and

the FFI method to produce sharp images of each antigen's pattern within embryos (T. Karr and T. Kornberg, in prep.). Each protein's pattern appears as a gray-to-black quenched zone against a background of bright, unquenched nuclei. We are able to map precisely the position of cells containing one, none, or both of the products examined in each embryo. By double-labeling embryos with the appropriate concentrations of three different pair-wise combinations of *hairy*, *ftz*, and *eve* antibodies, we can determine their relative spatial patterns. As *hairy* protein reaches its maximum level, it can be seen that there are cells that express both *hairy* and *ftz* (Fig. 2d). This has also been observed for their respective transcripts (Ingham et al. 1985). These cells are the most posterior cells within a portion of each *ftz* stripe. The overlap is more pronounced at ventrolateral positions (Fig. 2e, arrows) and is very transitory: By the time gastrulation is evident, *hairy* expression abuts but no longer overlaps *ftz* (Fig. 2f). Based on examination of a large sample of double-labeled cellular blastoderm- and early gastrulation-stage embryos, we conclude that accumulation of *hairy* within the more posterior *ftz*-containing cells leads quickly to repression of *ftz* expression and the observed narrowing of the *ftz* stripes (Carroll and Scott 1985).

The periodicity of *ftz* and *eve* can be used to extend the above observations and establish a more precise map of pair-rule protein spatial patterns. Double-labeling with *ftz* and *eve* antibodies shows that at the end of cellularization, the *eve* and *ftz* domains are often, but not always, separated by stripes approximately one cell wide (Fig. 2g,h); these gaps become more pronounced as gastrulation proceeds (Fig. 2i). This is largely in agreement with previous studies showing that *eve* defined the boundaries of odd-numbered parasegments, whereas *ftz* established the even-numbered parasegments (Frasch and Levine 1987; Lawrence et al. 1987).

Although *hairy* expression leads to repression of *ftz*, coexpression of *hairy* and *eve* is permitted. The *hairy* and *eve* protein patterns overlap extensively but are out of phase (Fig. 2j). At ventrolateral positions, *hairy* expression extends about three cells anterior to the first (most anterior) *eve* stripe, about two cells anterior to the second *eve* stripe, about one cell anterior to the third *eve* stripe, and appears to be virtually coincident with the fourth *eve* stripe, (Fig. 2k, arrow). Thus, although *hairy* exhibits a periodic pattern, it is out of phase with both the *ftz* and *eve* periodicity, and each *hairy* stripe varies subtly in intensity, width, and distance from adjacent stripes.

By comparing the above double- and single-labeling patterns of many individual staged embryos, the spatial relationships between all of these pair-rule products can be determined. We summarize our assessment of the relative spatial expression of the *hairy*, *ftz*, and *eve* proteins over a portion of the cellular blastoderm- and early gastrulation-stage embryo in Figure 3. It should be emphasized that earlier and later patterns exist that differ from the map in Figure 3. However, we have chosen to focus on the patterns as the embryo completes cellularization

Figure 2. The relative spatial patterns of the *hairy*, *ftz*, and *eve* pair-rule proteins. Embryos were stained with affinity purified rabbit antibodies to either the *hairy*, *eve* (courtesy of M. Frasch and M. Levine), or *ftz* proteins, singly or in pair-wise combinations and their patterns revealed by counterstaining with DAPI (T. Karr and T. Kornberg, in prep.). Anterior is to the left, ventral at the bottom of each image. (a) The wild-type *hairy* pattern. The seven transverse black stripes are separated by six unquenched white interstripes. (b) The wild-type *ftz* pattern. The seven quenched (black) stripes form around six unquenched (white) interstripes [*hairy* expression extends over most, but not all, cells within the interstripes (see d below)]. (c) The wild-type *eve* pattern. The seven quenched stripes are out of phase with the *ftz* stripes, and form one segment anterior to each *ftz* stripe (see g below) and partially overlap the *hairy* stripes (see j below). (d,e) The combined *hairy* and *ftz* protein patterns at low (d) and high (e) magnification. Nine zones of partial quenching are detectable, eight of which are transverse bands. The most anterior transverse band is made up of *hairy* protein alone. A band, about six cells wide, is detected posterior to a gap of about two to three cells. There are three levels of staining observed within this quenched zone. The staining is due to *ftz*-expressing cells on the anterior edge (brackets in e), it is due to *hairy*-expressing cells on the posterior edge (brackets in e), and there is a narrow discontinuous band of strongly quenched cells that express both *hairy* and *ftz* at the interface (arrowheads in d and e). This pattern is reiterated down the anteroposterior axis of the embryo six times until the most posterior band of *ftz* expression is reached. (f) Ventral view of an embryo showing the relationship between *hairy* and *ftz* expression at gastrulation. The weak *hairy* (h) stripes abut the stronger *ftz* (f) stripes but do not exhibit any detectable overlap at this stage. (g) The combined *ftz* and *eve* patterns establish 14 dark zones of quenching, which are interrupted in certain places by narrow strips of relatively unquenched bright nuclei (arrowheads). From the anterior (left), all gray zones are *eve* (e) domains, and all dark zones are *ftz* (f) domains. (h) High-magnification view of a portion of the embryo shown in (g). The *ftz* (f) and *eve* (e) zones are indicated. The arrowheads indicate the position of the same unquenched nuclei shown in g. (i) The relationship between *ftz* and *eve* at gastrulation. The *ftz* and *eve* stripes are narrower, and unquenched zones now separate each *eve* and *ftz* domain, as opposed to the slightly earlier stage shown in g; (f) *ftz* zones; (e) *eve* zones. (j) The combined *hairy* and *eve* patterns. Eight zones of quenching are created by the combined probes, seven of which are transverse bands. Each band is made up of an *eve* stripe that is partially overlapped by a stripe of *hairy* protein. At the anterior the first *hairy* stripe extends two to three nuclei anterior (bracket, h) to the first *eve* stripe (arrowhead). Although we can deduce that *hairy* overlaps *eve*, the very high relative level of *eve* expression precludes the detection of distinct overlap zones. The weakly quenched *hairy*-containing cells can be observed anterior to the first three *eve* stripes but extend anterior to them to different degrees. The most anterior *hairy* stripe does not extend completely ventrally; this is observed in about 10% of wild-type embryos. (k) High-magnification view of portion of the embryo shown in j. The brackets indicate the position of cells containing *hairy* only; the arrowhead indicates the position of an *eve* stripe that overlaps *hairy*.

Figure 3. Summary of relative spatial patterns of *hairy*, *ftz*, and *eve* at the completion of cellularization. From the inspection of embryos comparable or identical to those illustrated in Figure 2, the relative spatial patterns of *hairy* (orange), *eve* (purple), and *ftz* (green) expression are plotted. A schematic representation of the anterior ventrolateral portion of a cellular blastoderm-stage embryo (A) and the posterior ventrolateral portion of a gastrulating embryo (B) depicts the expression of the three proteins over the cells (circles) from a few segment primordia. The different parasegments (PS) represented are indicated. The *hairy* and *ftz* patterns have been traced directly from photomicrographs and the *eve* pattern has been derived from analysis of several multiply labeled embryos. Cells that express none of the three proteins are denoted by blue circles; overlaps between two protein patterns are indicated by the double coloring of circles.

and begins gastrulation, because it is the moment of high-level accumulation of each protein in its most complete pattern and best reflects the regulatory interactions believed to occur between these genes. The most pertinent observations schematized in Figure 3 are that (1) *hairy* and *ftz* proteins overlap transiently in the posterior-most cells of each *ftz* stripe; (2) *hairy* and *eve* proteins coexist at high levels in the anterior-most cells of each *eve* stripe; (3) these relationships evolve rapidly as the *ftz* and *eve* stripes narrow during cellularization and gastrulation; and (4) the exact spatial phasing of each stripe, especially the *hairy* pattern, is not identical within each double-segment unit. This last point was somewhat unexpected as we and others have treated each double-segment primordia and stripe of pair-rule gene expression as being largely equivalent. The subtle differences in pair-rule stripe widths and overlaps that

we observed as the embryo completes cellularization probably involve at least three processes. First, the *hairy* stripes appear asynchronously during cellularization; the regional differences in protein accumulation probably reflect gap gene influences that differ greatly between segment primordia. Second, the *eve* and *ftz* patterns narrow as cells enter the ventral furrow and transverse folds begin to form, and the density of cells in the ectoderm changes, so some of these phase changes may reflect cell movements. And, finally, the decay of individual protein stripes may occur at different rates depending upon the extent of active repression occurring within individual cells and the rate of protein turnover. The mechanism for turning off pair-rule gene expression is not known; it is likely that it is also a significant factor in the phase shifts in gene expression patterns in different segment primordia.

Regulation of the *ftz* gene by the *hairy* protein

It has been shown previously that the zygotic loss of *hairy*-gene function leads to an expansion in the *ftz* expression domain (Carroll and Scott 1986; Howard and Ingham 1986). We can compare how *ftz* expands in a *hairy*⁻ embryo with the normal domains of *hairy* and *ftz*. In wild-type embryos, the sum of *hairy* and *ftz* expression still leaves seven narrow bands of unlabeled cells (Fig. 4a). However, as shown in Figure 4b, in *hairy* mutant embryos that fail to express detectable *hairy* protein (not shown), *ftz* expression expands into broad

Figure 4. *ftz* expression in *hairy* mutants extends into cells not normally accumulating *hairy* to high levels. Embryos were stained with antibodies, as described in Figure 1. (a) Wild-type embryo at the cellular blastoderm stage doubly labeled with *ftz* and *hairy* antibody. Note the seven narrow bands of unquenched nuclei (lines) separating each broader domain; these cells express neither *ftz* nor *hairy* protein. (b) The expression of *ftz* in an embryo at the same stage as in a, but this animal is homozygous for the strong *hairy*^{7H94} allele. *ftz* expression expands into broad largely continuous domains that include the normal *ftz*-expressing cells, cells normally expressing *hairy*, and cells normally at the unlabeled boundaries indicated in a (lines). We analyzed 25 homozygous *hairy*^{7H94} embryos, and they all exhibited the broad *ftz* pattern.

domains that are larger than the domains defined by the sum of *hairy* and *ftz* expression in wild-type embryos. These results support the view that *hairy* is normally required in cells that do not accumulate it to high levels (Howard and Ingham 1986). We have observed transient early patterns of *hairy* protein accumulation in cells that do not belong to any of the final cellular blastoderm *hairy* stripes (S. Carroll, unpubl.), perhaps these cells require *hairy* transiently for normal *ftz* regulation. Thus, unstable intermediate *hairy* expression patterns, rather than cell nonautonomy (Howard and Ingham 1986), may explain the behavior of *ftz* expression in *hairy* mutant embryos.

The correlation between *hairy* and *ftz* expression in gap mutants

The zygotically required gap genes *Krüppel* (*Kr*), *hunchback* (*hb*), and *knirps* (*kni*) affect the development of large regions of the embryo. Loss of each of their functions leads to deletions of continuous blocks of segments (Nüsslein-Volhard and Wieschaus 1980) and improper expression of pair-rule genes (Carroll and Scott 1986; Ingham et al. 1986; Frasch and Levine 1987). It is not known at what level gap genes influence pair-rule gene expression, which interactions are direct or indirect, or how the patterns of gap gene expression are related to the periodic pair-rule patterns. One step toward analyzing gap gene/pair-rule gene interactions is to understand how each gene is affected by the absence of gap gene function. In a previous study, it was shown in *Kr* mutant embryos that *hairy* and *ftz* transcript patterns assume complementary patterns, indicating that the effect of *Kr* upon *ftz* may be mediated indirectly through *hairy* (Ingham et al. 1986).

The *ftz* gene is expressed abnormally in embryos lacking *Kr*⁺ (Fig. 5a), *kni*⁺ (Fig. 5c), or *hb*⁺ (Fig. 5e) functions. To determine whether any of these effects could be mediated through the regulatory action of the *hairy* gene, we examined *hairy* expression in these same mutants (Fig. 5b,d,f). In the *Kr*⁻ and *kni*⁻ embryos, the *ftz* patterns are complementary to the *hairy* patterns (Fig. 5a–d, arrows indicate complementary regions). Because *hairy* regulates *ftz*, but *ftz* has no influence on *hairy* (Howard and Ingham 1986), it is evident that the effects of these two mutants on *hairy* and *hairy*'s negative control of *ftz* may largely explain the observed *ftz* patterns. However, in the *hb*⁻ embryo, the *ftz* and *hairy* patterns are not entirely complementary (Fig. 5e, f). In the posterior region of the *hb*⁻ embryo, *hairy* and *ftz* expression expand into broader domains (Fig. 5e,f, brackets). The seventh *hairy* stripe overlaps the fused sixth and seventh *ftz* stripes. Normally, we would expect that, in its role as a negative regulator of *ftz*, *hairy* would prevail and repress *ftz* expression over this region. This is not the case in *hb*⁻ embryos; the broad posterior *ftz* stripe persists through gastrulation. It appears then that *hb* must be controlling more than just *hairy* to explain its effects on *ftz*. *hb* could act directly on *ftz* or control yet another gene that directly regulates *ftz*. It is known that *run* and *eve* also regulate *ftz* (Carroll and Scott 1986) and that they interact with each other and *hairy* (Frasch and Levine 1987; S. Carroll, unpubl.). The complete elucidation of the early segmentation gene network will require detailed study of these subtle and frequently bilateral interactions. Clearly, further investigation into the mechanism of regulation of the *hairy* gene by the gap genes and the other pair-rule loci is intimately tied to understanding how *ftz* comes to be expressed in stripes.

Figure 5. *hairy* expression in gap mutants is complementary to that of its regulatory target, the *ftz* gene. Embryos homozygous for strong mutations at the *Kr*, *kni*, and *hb* gap loci were stained with either *ftz* (a,c,e) or *hairy* (b,d,f) antibody. (For the wild-type patterns, see Fig. 2a,b.) The correlations between each spatial pattern were confirmed by double labeling mutant embryos with *hairy* and *ftz* antibodies (not shown). (a,b) *ftz* and *hairy* patterns in *Kr*¹ embryos. The arrows indicate how regions that express *ftz* do not express *hairy*. (c,d) *ftz* and *hairy* patterns in *kni*¹⁴⁷² embryos. The arrows indicate the complementarity between the patterns. (e,f) *ftz* and *hairy* patterns in *hb*⁻ embryos. The patterns are largely, but not entirely, complementary. Posteriorly it appears that *ftz* and *hairy* overlap over a significant length of the embryo, (see posterior bracket). The *ftz* pattern is unexpectedly stable and is not fully repressed by the expanded *hairy* stripe.

The function of the nuclear hairy protein

The nuclear localization of *hairy*, its spatial relationship to *ftz* expression, and the pattern of *ftz* expression in *hairy* and gap mutant embryos all suggest a regulatory role for *hairy* in the control of *ftz* gene expression. In addition, it has been shown recently that induction of *hairy* expression throughout the embryo utilizing a heat shock promoter-*hairy* gene construct results in the elimination of *ftz* expression (Ish-Horowicz and Pinchin 1987). These observations are all consistent with, but do not yet prove, a direct function for *hairy* in regulating *ftz* gene expression.

The *hairy* protein could interact with *ftz* DNA, RNA, or protein. It is doubtful that *hairy* interacts solely at the RNA level because it has been shown that the turnover of *ftz* RNA takes place at equivalent rates across the embryo, in both the *hairy*-containing interstripes and in the *ftz* stripes lacking *hairy* expression (Edgar et al. 1986). *hairy* could indeed be a transcriptional regulator, but it is important to note that unlike *ftz*, *eve*, and a variety of other *Drosophila* genes, *hairy* does not contain a homeo box (for review, see Scott and Carroll 1987), nor does it contain a nucleic acid-binding zinc finger structure present in the Krüppel (Rosenberg et al. 1986) and *hb* (Tautz et al. 1987) gap genes (C. Rushlow, pers. comm.). If *hairy* acts via binding to nucleic acid, it utilizes a different mechanism than that of other characterized *Drosophila* regulatory genes. All data are also consistent with a potential function for *hairy* in inactivation of the *ftz* protein. The full-level activation of the *ftz* gene involves an auto-feedback enhancement by the *ftz* protein (Hiromi and Gehring 1987). Inactivation by *hairy* of this autocatalytic mechanism at the *ftz* protein level could be one way to regulate the pattern of *ftz* expression. In addition, it has been shown that *hairy* may be a positive regulator of *eve* (Frasch and Levine 1987). The determination of the biochemical function of the *hairy* protein and its potentially opposite effects on different pair-rule genes await in vitro analysis of these various possibilities with purified recombinant protein.

Methods

Antibodies

Rabbit antibodies to the *eve* protein were generously provided by M. Frasch and M. Levine. The *ftz* and *hairy* antibodies were produced as described elsewhere (Carroll and Scott 1985; Carroll and Laughon 1987).

Immunolabeling

Embryos were permeabilized, fixed, devitellinized, and prepared for staining as described elsewhere (Carroll and Scott 1985).

Primary antibodies were incubated at 0.1–1.5 µg/ml in phosphate-buffered saline containing 10 mg/ml bovine serum albumin (BSA) and 0.1% Triton X-100 (vol/vol). The antibodies were detected by incubation with biotinylated goat anti-rabbit IgG followed by streptavidin–horseradish peroxidase conjugate and development with diaminobenzidine in the presence of 0.02% H₂O₂ and 0.03% Co²⁺ and Ni²⁺. Embryos were then

counterstained with DAPI at 10 µg/ml for 30 min and mounted for viewing in 50 mM Tris (pH 8.8) containing 10% glycerol (vol/vol).

Bright-field photography was done with Kodak Technical Pan film, and fluorescence photography was done with Kodak Tri-X film.

Double labeling with rabbit antibodies was achieved by careful titration of the relative concentrations of each reagent.

Detailed protocols are available upon request.

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